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Catalysis and Biocatalysis Program

Final Report

J. D. Ingham

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Prepared for

Energy Conversion and Utilization Technologies Division
Office of Energy Systems Research
and
Advanced Industrial Concepts Division
Office of Industrial Technologies
U.S. Department of Energy

Through an agreement with

National Aeronautics and
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Jet Propulsion Laboratory
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This report covers work performed for the Catalysis and Biocatalysis Program within the United States Department of Energy from May 1980, through December 31, 1991. The Program was under the direction of DOE Program Management with the Jet Propulsion Laboratory, California Institute of Technology, serving as field manager for the United States Department of Energy through an agreement with the National Aeronautics and Space Administration (NASA Task RE-152, Amendment 307; DOE Interagency Agreement DE-A101-86CE90239).

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FOREWORD

The Advanced Industrial Concepts Division (AICD) Biological and Chemical Technologies Research (BCTR) Program evolved from the Energy Conversion and Utilization Technologies (ECUT) Projects/Programs in the chemical processes, catalysis, and biocatalysis areas. There have been a number of transitions in the 12 years since the initiation of the Program in May, 1980. The ECUT Chemical Processes Project was established at that time, and the Jet Propulsion Laboratory, California Institute of Technology, was chosen as the lead laboratory for the Project through an interagency agreement between NASA and DOE. The initial goal was to develop a technology base for more efficient production of industrial chemicals, with activities in two technical areas: catalysis (chemical and biocatalysis) and separations. In 1983, still within ECUT, the program became known as the Biocatalysis Research Activity, reflecting a continuation of only the biocatalysis component of the program. From 1984 through 1989 it was known as the Biocatalysis Project and consisted of three technical work elements: Molecular Modeling and Applied Genetics, Bioprocess Engineering, and Process Design and Analysis.

Technical efforts in each work element were implemented that would contribute to an expanded biotechnology base for processes to be developed for the efficient production of high-volume, low-cost chemicals from renewable resources. In early 1990, with an increased interest in novel chemical processing, the program was expanded to include chemical catalysis and was subsequently renamed the Catalysis and Biocatalysis Program. The overall Program was modified to include five work elements: Molecular Modeling and Catalysis by Design, Applied Microbiology and Genetics, Bioprocess Engineering, Separations and Novel Chemical Processes, and Process Design and Analysis. In April of 1990, a reorganization of the Office of Conservation and Renewable Energy (CE) moved this program to the AICD under the Office of Industrial Technologies and the Office of Industrial Processes (OIP).

With the onset of Fiscal Year 1992, the AICD Biocatalysis Program again experienced a period of transition. The transition reflects a need for the Program to assume a greater R&D role in chemical catalysis as well as a need to position itself for a more encompassing involvement in a broader range of biological and chemical technology research.

As part of the transition process, the Program title and structure have changed. The new Program title is the Biological and Chemical Technologies Research (BCTR) Program, for which there are five Program elements: Catalysis-by-Design, Advanced Bioprocessing Systems, Novel Process Systems, Feedstock/Process Interactions, and Process Control and Systems Analysis.

The transition is not intended to alter the overall goals of the

Program. In fact the new structure, in alignment with the National Energy Strategy (NES) is intended to bolster the Program toward its objectives to:

1. Provide the technology base in development of new chemical and biological approaches for chemicals production that are more economically competitive, less energy intensive, and environmentally sound.
2. Facilitate the introduction of renewables into the chemicals industry.
3. Enhance and expand the application of biotechnology in the chemicals industry through integrated or new process operations.
4. Provide technological support in response to industry and other CE program R&D needs as they pertain to energy efficiency, economic viability, and environmental safety.

Through the planning, initiation, execution, and successful completion of its R&D activities, the Program will communicate the results of these research activities to the industrial sector for effective technology transfer. In keeping with tradition, the Program will foster R&D through cooperative research ventures with industry and through integration of research activities with the CE applied activities. Since this report concludes with the transition of the Catalysis and Biocatalysis Program to the Biological and Chemical Technologies Research Program, there is no discussion of the research conducted within the BCTR Program.

For further information about the AICD Biological and Chemical Technologies Research Program, contact:

David J. Boron
United States Department of Energy
Advanced Industrial Concepts Division
Office of Industrial Technologies
CE-232, Rm 5F-043
1000 Independence Avenue SW
Washington, DC 20585
(202) 586-0080

or

Dr Gene R. Petersen
National Renewable Energy Laboratory
1617 Cole Boulevard
Golden, CO 80401
(303) 231-1984

ABSTRACT

This final report presents a summary of research activities and accomplishments for the Catalysis and Biocatalysis Program, which has been renamed the Biological and Chemical Technologies Research (BCTR) Program, currently of the Advanced Industrial Concepts Division (AICD), Office of Industrial Technologies of the Department of Energy (DOE). The Program was formerly under the Division of Energy Conversion and Utilization Technologies (ECUT) until the DOE reorganization in April, 1990. The goals of the BCTR Program are consistent with the initial ECUT goals, but represent an increased effort toward advances in chemical and biological technology transfer. In addition, the transition reflects a need for the BCTR Program to assume a greater R&D role in chemical catalysis as well as a need to position itself for a more encompassing involvement in a broader range of biological and chemical technology research. The mission of the AICD is to create a balanced Program of high risk, long-term, directed interdisciplinary research and development that will improve energy efficiency and enhance fuel flexibility in the industrial sector. Under AICD, the DOE Catalysis and Biocatalysis Program sponsors research and development in furthering industrial biotechnology applications and promotes the integrated participation of universities, industrial companies and government research laboratories.

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FINAL REPORT

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SECTION I

EXECUTIVE SUMMARY

This Final Report is a summary of research activities and accomplishments for the Catalysis and Biocatalysis Program, which is now known as the Biological and Chemical Technologies Research (BCTR) Program. The BCTR Program is currently under the Advanced Industrial Concepts Division (AICD), Office of Industrial Technologies of the Department of Energy (DOE). The Program was formerly under the Division of Energy Conversion and Utilization Technologies (ECUT) until the Department of Energy reorganization in April, 1990. The mission of the AICD is to create a balanced Program of high-risk, long-term, directed interdisciplinary research and development that will improve energy efficiency and enhance fuel flexibility in the industrial sector. Under AICD, the DOE Catalysis and Biocatalysis Program sponsors research and development in furthering industrial biotechnology applications and promotes the integrated participation of universities, industrial companies and government research laboratories.

In May, 1980 the ECUT Chemical Processes Project was established and the Jet Propulsion Laboratory, California Institute of Technology, was chosen as the lead laboratory for the Project through an interagency agreement between NASA and DOE. The initial goal was to develop a technology base for more efficient production of industrial chemicals. Activities of the Project are shown in Table 1. From May, 1980 to July, 1981 JPL assisted ECUT in the evaluation and prioritization of potential research areas. These planning studies resulted in the selection of activities in two technical areas: catalysis (chemical and biocatalysis) and separations. In 1981 two research tasks from Caltech in the Department of Chemistry and Chemical Engineering were initiated: "Microscopic Reaction Models for Catalytic Processes", W.A. Goddard, III; and "Influences of Host-Cell-Plasmid Interactions on Productivity of Recombinant Microorganisms", J.E. Bailey. The first one involved modeling Fischer-Tropsch catalytic processes, which have been used commercially for the production of hydrocarbons, alcohols and other chemicals from synthesis gas (mixtures of carbon monoxide and hydrogen); and the second was directed toward optimization of reactivity and stability of recombinant microorganisms.

The 1982 Chemical Processes Project had the same work elements (Catalysis and Separation) as in 1981. In 1983 the Project was discontinued as a result of a major reorganization of all ECUT projects, and was configured as the Biocatalysis Research Activity with two work elements: Biocatalysis and Molecular Modeling.

From 1984 through 1989 the Activity became known as the Biocatalysis Project and contained three technical work elements: Molecular Modeling and Applied Genetics, Bioprocess Engineering,

and Process Design and Analysis. These emphasized specific technical areas according to scale dimensions. The modeling element was directed to research activities at the molecular and cellular levels (one micron or smaller). Research at this level defines molecular interactions and is the basis for reactor design and applications. The bioprocess work element defines engineering relationships to take advantage of molecular level results, e.g., as a basis for new reactor design concepts at dimensions of the order of one meter. The process design work element involves activities at the commercial process level at dimensions of hundreds of meters, and provides energy and economic assessments, as well as mechanisms for the transfer of technology to the industrial sector. The three work elements provide an orderly system for progress from a basic understanding of events at the molecular level, such as biocatalyst reactivity and stability, to macro-level effects on reactor operations, and then to the development and evaluation of improved processes with respect to economic advantages and energy efficiency.

Technical efforts in each work element were implemented that would contribute to an expanded biotechnology base for processes to be developed for the efficient production of high-volume, low-cost chemicals from renewable resources. In 1990, the name was changed to the Catalysis and Biocatalysis Program. As a result of reorganizations in the Office of Conservation and Renewable Energy, the Program was moved to the Advanced Industrial Concepts Division (AICD), Office of Industrial Technologies in April, 1990. Five work elements were included: Molecular Modeling and Catalysis by Design, Applied Microbiology and Genetics, Bioprocess Engineering, Separations and Novel Chemical Processes, and Process Design and Analysis.

The onset of Fiscal Year 1992 found the AICD Biocatalysis Program in a period of transition. The transition reflects a need for the Program to assume a greater R&D role in chemical catalysis as well as a need to position itself for a more encompassing involvement in a broader range of biological and chemical technology research.

As part of the transitioning process, the Program title and structure have changed. The new title is the Biological and Chemical Technologies Research Program (BCTR), for which there are five Program elements: Catalysis-by-Design, Advanced Bioprocessing Systems, Novel Process Systems, Feedstock/Process Interactions, and Process Control and Systems Analysis.

Catalysis-by-Design (CBD).

The catalysis-by-design activity was initiated in 1989 with the objective to develop a theoretical predictive base to guide the search for new, more efficient catalysts. The work is carried out by federal laboratories, universities, and industry, working

cooperatively on non-proprietary aspects of technology (simulations and model development). The work uses prototype systems and provides theoretical computational models (or parts thereof) such that industry can increase its efficiency in the design of catalysts.

The objective of the Catalysis-by-Design Program element is to develop modeling tools to aid in the design of chemical and biological catalysts in support of new chemical and material product-line and process-route developments.

Advanced Bioprocess Systems (ABS).

The ABS addresses research in process biochemistry and bioprocess engineering and manufacturing related to the production of chemicals and materials. The objective of the Advanced Bioprocess Systems Program element is to develop and evaluate novel bioprocessing approaches for the production of chemicals and materials.

Two general R&D approaches are being pursued within this effort; (1) the direct integration of bioprocessing into the chemicals industry, and (2) the production of chemical products from non-petroleum feedstocks. To facilitate the introduction of biotechnology in the chemicals industry, research is focusing on the development of biocatalysts and bioprocesses that operate in nonaqueous media (so that bioprocesses can be integrated directly into existing process operations). Conversely, aqueous-phase bioprocessing is being developed for the purpose of producing chemicals from indigenous energy sources. In some cases, these processes may not necessarily be integrated directly into the current chemical industry, but may be used at smaller grassroots facilities, e.g., as plant operations to efficiently supplement agricultural operations that utilize renewable resources.

Novel Process Systems (NPS).

Opportunities exist to develop biotechnologies for niche market application or in replacement of conventional process approaches. In a similar manner, there are opportunities to pursue novel chemical approaches that hold promise for more efficient operation (e.g., improved "production intensity," lower "energy intensity," etc.) than existing commercial processes.

The objective of the Novel Process Systems Program element is to explore innovative biological and chemical approaches related to the development of novel chemical syntheses and processes. This Program element provides for exploratory research in the development of novel biological or chemical approaches that may lead to improved industry process operations in energy efficiency,

productivity, and/or environmental soundness. As exploratory R&D ventures, each project is envisioned as a two-to-five year research effort. The information generated should identify research opportunities for continued development of innovative processes and process syntheses, or for the production of new chemical products. Research activities include novel chemical and biological process syntheses, separation approaches, recovery systems, etc., related to chemicals production.

Feedstock/Process Interaction (FPI).

For most mature chemical processes, feedstock costs constitute about 70% of the total manufacturing cost and about 30% of the overall energy consumption. The combined impact of feedstocks on product cost and energy is higher than any other process component.

The objective of the Feedstock/Process Interaction Program element is to develop the technology base for the design of biological or chemical approaches (or combinations thereof) that will facilitate the introduction and expanded use of renewables and recoverables as feedstocks to displace petroleum in the production of chemicals. The research is intended to identify opportunities to reduce process costs, maximize feedstock utilization, and minimize waste generation in the utilization of alternative resources.

Alternative feedstocks, e.g., lignocellulosic material, some plastics, etc., are not amenable to conventional processing, and at the moment, their utilization would lead to very high processing costs. Successful R&D should provide the chemical industry with greater feedstock flexibility in the production of chemicals.

Process Control and Systems Analysis (PCSA).

The successful development and deployment of advanced chemical and biological technologies can be facilitated by effective process design, operation, and economic models to guide research and development efforts. The objective of the Process Control and Systems Analysis Program element is to develop process model and simulation capabilities to guide process scientists and engineers in the development of new chemical and biological technologies, and to improve process control systems to help operations engineers maintain optimum performance of advanced process operations.

Through 12 years in exploratory and applied research, the BCTR Program and its predecessors have made significant contributions in the development of biological and chemical approaches to produce chemicals from alternative energy sources. Selected Program accomplishments include:

- The successful engineering of biocatalysts that

facilitate novel reaction syntheses in organic media.

- The development of process simulation models, such as the Bioengineering Simulation Technology (BEST) and the Biological and Chemical Process (BCP) models, that simulate advanced chemical and biological processes.
- The development of conceptual designs for advanced bioprocess systems and approaches, such as the biosolubilization of phosphate.
- The development of modeling tools for characterizing and describing chemical catalysts.
- The design and engineering of advanced bioreactors, such as the biparticle fluidized-bed reactor, that have higher yields and productivities than conventional fermentation systems.
- The formation of a state-of-the-art research center located at the California Institute of Technology to promote and facilitate government-industry-university interaction and collaboration.

The Program maintains strong industrial participation and collaboration in its research activities. In 1992, the Program maintained a constituency of 33 industrial partners that contributed approximately \$3.5 million in joint research efforts.

SECTION II

PROJECT ACTIVITIES

A summary of Project Activities from 1981 through 1991 is shown in Table 1, and more detailed descriptions are provided in the following subsections.

A. Chemical Processes (1981-1982)

Planning studies (May, 1980 to mid-1981) resulted in the selection of activities in two technical areas: catalysis (chemical and biocatalysis) and separations. In 1981 two research tasks from Caltech in the Department of Chemistry and Chemical Engineering were initiated: "Microscopic Reaction Models for Catalytic Processes", W.A. Goddard, III; and "Influences of Host-Cell-Plasmid Interactions on Productivity of Recombinant Microorganisms", J.E. Bailey. The first one involved modeling chemical catalytic processes that have been used for the production of hydrocarbons, alcohols and other chemicals. The second was directed toward optimization of reactivity and stability of recombinant microorganisms.

In FY 1982 there was also an extensive planning activity which is described below under Planning Support. The ECUT Program Manager was Dr. J.J. Eberhardt at the Department of Energy, and the Project Manager was Dr. J. Moacanin at the Jet Propulsion Laboratory.

Catalysis-Catalyst Modeling

During FY 1982 the Catalysis work element included four research activities in catalyst modeling: (i) Microscopic Reaction Models for Catalytic Processes; (ii) Electrocatalysis; (iii) Decarboxylation; and (iv) Ammonia Synthesis.

(i) Microscopic Reaction Models for Catalytic Processes, W.A. Goddard, Caltech This involved modeling Fischer-Tropsch (FT) catalytic processes, which have been used commercially for the production of hydrocarbons, alcohols and other chemicals from synthesis gas (mixtures of carbon monoxide and hydrogen). The problems with this type of process are a lack of selectivity and limited understanding of catalytic mechanisms. Accomplishments included: development of calculations of force fields and dynamics of catalysis which lead to initial conclusions that stabilization of group VIII metal formyls is not critical in FT catalysis; a determination that certain types of reactive species (analogous to those involved in other types of reactions) are not likely; and that the modeling of the energetics of FT processes on nickel clusters suggests that there may be a lower energy for propagation than for methane production (which is consistent with formation of larger amounts of higher hydrocarbons with nickel catalysts).

(ii) Electrocatalysis, L.F. Warren, Rockwell International Microelectronics The objectives were to (a) evaluate areas where electrocatalysis could result in significant energy conservation, and (b) to perform preliminary research on electrocatalysis for energy reduction. Accomplishments: (a) areas identified were inorganic synthesis of chlorine, aluminum and hydrogen; organic synthesis, as adiponitrile produced at a scale of >400 million lb/y; and electrochemical energy production and storage. (b) A promising oxygen electrocatalyst, lanthanum nickelate, was synthesized.

(iii) Decarboxylation, S. Di Stefano, A. Gupta, and J.D. Ingham Jet Propulsion Laboratory The objectives were to develop decarboxylation catalysts to convert carboxylic acids in dilute aqueous waste streams, or as fermentation products, to hydrocarbons, which would reduce pollutant levels and provide useful products from wastes or renewable resources. Accomplishments: a rhodium-based catalyst was prepared which appeared to decarboxylate acetic acid at 1-10 vol%, but further work was needed to determine kinetics, yields and susceptibility to inhibition; and examined the use of electron spin resonance (esr) spectroscopy and electrochemistry for mechanistic studies.

(iv) Ammonia Synthesis, G.E. Voecks, Jet Propulsion Laboratory The industrial process was investigated to establish potential energy savings which could result from improving the conversion efficiency of the catalyst. Accomplishments: results indicated that improved catalysts could result in a 30% increase in thermal efficiency and substantial energy savings.

Catalysis-Biocatalysis

This work element included four activities: (i) Kinetics for Process Design; (ii) Techniques for Plasmid Monitoring; (iii) Cellulase Hyperproduction; and (iv) Membrane Fouling.

(i) Kinetics for Process Design, J.E. Bailey, Caltech The objective was to model genetically engineered microorganisms and determine how to control plasmid replication to develop stable, more efficient, selective biocatalysts. Accomplishments: An initial model was formulated for predicting the effects of genetically engineered traits on protein production levels in the bacterium E. coli.

(ii) Techniques for Plasmid Monitoring, J.E. Bailey, Caltech Recombinant DNA technology allows the design of microorganisms that are tailored to produce chemical products or enzymes on demand. This is done by introducing genetic instructions in the form of plasmids into the microorganism. Unfortunately, on dividing, the cell may lose some of these plasmids in successive generations, as well as the corresponding desired traits, such as the ability to produce the desired product. The objective of this work was to develop a method for plasmid monitoring. Accomplishments: A

microfluorometric method (where the plasmid-encoded enzyme interacts with a fluorogenic substrate and its fluorescence is measured) was developed to measure plasmid contents in cells.

(iii) Cellulase Hyperproduction, G.A. Nelson, Jet Propulsion Laboratory The objectives were to genetically alter the metabolic regulation of cellulolytic fungi to increase cellulase synthesis rates and to augment enzyme secretion rates to decrease separation costs. (Cellulase enzymes are very important because they are the biocatalyst that converts cellulose to glucose, and if they were more readily available, many bioprocesses would be economically competitive.) Accomplishments: assay procedures to identify hyperproducing and nonproducing fungus mutants and to measure cellulase activity were developed; two genetically manipulatable cellulase producers were identified from 18 strains that were screened.

(iv) Membrane Assessment, J.D. Ingham, Jet Propulsion Laboratory Because membrane processes require much less energy for product separation than most alternatives (such as distillation), it was suggested that they may be effective for separation or concentration of fermentation products; however, earlier experiments with some biological solutions indicated that severe problems could arise from membrane fouling and concentration polarization. The purpose of this work was to review previous work on membranes to provide a preliminary assessment of membrane processes for separation of products from biocatalyzed processes. Accomplishments: The review concluded that (a) membrane processes are mainly applicable to increase product concentrations from a few wt% to <40 wt%; (b) in many cases the effects of concentration polarization and fouling, and the fact that the diluent (present in much larger amounts than the product) must normally pass through the membrane instead of the product tend to limit the applications of membranes; (c) selective permeation of vapor (pervaporation) may be appropriate for some fermentation products, but energy as the heat of vaporization is still consumed, and suitable membranes would need to be developed.

Separation

There were three research activities: (i) Second Law Analysis; (ii) Membrane Technology; and (iii) Critical Fluid Extraction.

(i) Second Law Analysis, C. England, Engineering Research West The objective was to investigate energy conservation approaches that considered effects of the second law of thermodynamics, which generally leads to loss of energy because heat at a high temperature always flows in a direction toward a lower temperature. Accomplishments: several potential areas for energy conservation were identified, including recovery of waste heat, which is often discharged in cooling towers if the temperature is up to about

78°C; use of a membrane process for separation of oxygen from air; and recovery of energy in the pressure cycle if supercritical fluid extraction is used as a separation method.

(ii) Membrane Technology, H.K. Lonsdale, Bend Research, Inc. This study was undertaken to identify the potential applications of membrane processes to improve energy efficiency and to identify membrane research needs in areas of interest. Accomplishments: the study resulted in the conclusion that membranes could be used advantageously to separate carbon dioxide from gas streams, oxygen from air, and fermentation products from water; to recover heat from water vapor in drying processes; and to isolate immobilized biocatalysts. Recommended research areas included enhancement of membrane permeability and selectivity, facilitated transport mechanisms, and investigation of liquid membrane systems.

(iii) Critical Fluid Extraction, Critical Fluid Systems, Inc. Critical fluid extraction processes consist of the use of solvents above their critical pressure and temperature to separate the product. As a supercritical fluid, the solvent acts as a very dense gas that has desirable solution characteristics. The pressure is released after extraction and the product spontaneously separates from the vaporized fluid. The objective was to identify research areas for potential applications of supercritical fluid extraction. Accomplishments: areas identified included separations of oxygenated organic chemicals, vegetable oils, and fossil fuels; investigation of cosolvents to increase product concentrations; and studies of chemical reactions in supercritical fluids.

Planning Support

This activity included initiating a Guidance and Review Panel, an Energy and Economic Analysis, and preparation of Multi-Year Research Agenda.

(i) Guidance and Evaluation Panel, J. Moacanin, Jet Propulsion Laboratory This consisted of an advisory group from universities, Government agencies, and industrial companies. Accomplishments: the first panel was established and consisted of the following individuals: J.E. Bailey, Caltech; E. Dunlop, Washington University; J. Eberhardt, DOE; R. Gomez, Genentech; L. Kim, Shell Oil; J. Moacanin, JPL; and W. Weigand, NSF. Three priority areas for biocatalysis research were defined: increased product concentration, increased reaction rates, and increased size or altered structure of genetic information.

(ii) Energy and Economic Analysis, K. Stern, Chem Systems Inc. This activity consisted of an evaluation of the biocatalyzed process for acetone/butanol/ethanol (ABE). Accomplishments: it was established that because the maximum product concentration is so low (<2 wt%), separation energy requirements are so high (>20,000

btu/lb of solvents) that this is not a practical process. The problem is toxicity of product to the microorganism, which severely limits the rate of formation and concentration of bioprocess products.

(iii) Multi-Year Research Agenda, R. Wilcox, Jet Propulsion Laboratory This consisted of preparation of a report on future plans for catalyst modeling and biocatalyst research. Accomplishments: for catalyst modeling the Agenda describes a 10-year program for establishing the technical feasibility for theoretical design, optimization and control of chemical process catalysts, and for biocatalysis, a 15-year program on genetic engineering of microorganisms to provide for more efficient production of industrial chemicals.

B. Biocatalysis Research Activity (1983)

In 1983, ECUT was reorganized and work in the Chemical Processes Project continued as the Biocatalysis Research Activity with two technical work elements: Molecular Modeling and Biocatalysis. There was also a Planning Support activity which is also described below. The ECUT Program Manager was Dr. J.J. Eberhardt at the Department of Energy, and the Project Manager was Dr. J. Moacanin at the Jet Propulsion Laboratory.

Molecular Modeling

During FY 1983 this work element included two research activities in modeling: (i) Enzyme Reaction Models for Catalytic Processes, and (ii) Electrocatalysis.

(i) Enzyme Reaction Models for Catalytic Processes, W.A. Goddard, Caltech This research involved development of an enzyme catalysis model to describe the three-dimensional structure and molecular behavior in catalytic processes. The ultimate goal was to develop the capability to design new selective enzymes and enzymes for chemical conversions in nonaqueous media. Accomplishments: an initial force-field model including important molecular interactions was developed; this model was used successfully to relate calculated enzyme parameters to experimental observations and to examine the dynamic behavior of the enzyme thermolysin.

(ii) Electrocatalysis, L.F. Warren, Rockwell International Microelectronics Research Center The objectives were to (a) evaluate areas where electrocatalysis could result in significant energy conservation, and (b) perform preliminary research on electrocatalysis for energy reduction. Accomplishment: a promising electrocatalyst (based on molybdenum-phosphine bi-dentate ligand) that can be attached to graphite electrodes for the direct reduction of nitrogen was developed.

Biocatalysis

Biocatalysis consisted of four activities: (i) Kinetics for Process Design; (ii) Techniques for Plasmid Monitoring; (iii) Cellulase Hyperproduction; and (iv) Chromosomal Amplification.

(i) Kinetics for Process Design, J.E. Bailey, Caltech The objective was to model genetically engineered microorganisms and determine how to control plasmid replication to develop stable, more efficient, selective biocatalysts. Accomplishments: a previously formulated detailed molecular level mathematical model was used to investigate alternative molecular designs for regulation of plasmid functions to improve control with recombinant strains. Kinetic models were developed to calculate the influences of genetic instability in different bioreactor configurations.

(ii) Techniques for Plasmid Monitoring, J.E. Bailey, Caltech Recombinant DNA technology allows the design of microorganisms that

are tailored to produce chemical products or enzymes on demand. This is done by introducing genetic instructions in the form of plasmids into the microorganism. The objective of this work was to develop a method for plasmid monitoring. Accomplishments: (a) the sensitivity of the microfluorometric method (where the plasmid-encoded enzyme interacts with a fluorogenic substrate and its fluorescence is measured to monitor plasmids) was increased by a factor of 1000; and (b) new yeast strains were constructed to permit studies of the effects of plasmid replication controls to retain the plasmid and to synthesize proteins encoded on plasmid genes.

(iii) Cellulase Hyperproduction, G.A. Nelson, Jet Propulsion Laboratory The objectives were to genetically alter the metabolic regulation of cellulolytic fungi to increase cellulase synthesis rates and to augment enzyme secretion rates to decrease separation costs. Accomplishments: (a) two genetically manipulable cellulase producers were identified from 18 strains and exhibited evidence that cellulase is subject to catabolite repression; (b) studies with the drug chlorpromazine indicated that secretion may be under calmodulin/calcium regulation, which may provide a way to select secretion mutants that are resistant to calmodulin inhibitors.

(iv) Chromosomal Amplification, G.E. Bertani, Jet Propulsion Laboratory The purpose of this work was: (a) to demonstrate the feasibility of transfer and amplification of genetic material directly in the chromosome of a microorganism; and (b) to measure the genetic stability and compare it with a conventional genetically-engineered microorganism with genetic material carried by plasmids. (In this type of context, stability refers to retention of the genetic characteristic in succeeding generations.) Accomplishments: (a) chromosomal integration of appropriately engineered plasmids was inferred from genetic methods, and (b) the stability of the strains carrying an integrated plasmid was increased by using DNA from a bacteriophage mutant defective for integration.

Planning Support

This activity included the Guidance and Review Panel, an Energy and Economic Analysis, Second Law Analysis, and the Research Opportunity Notice (RON).

(i) Guidance and Evaluation Panel, J. Moacanin, Jet Propulsion Laboratory This consisted of a technical review board from universities, government agencies, and industrial companies. Accomplishments: the panel held a meeting during the AIChE meeting in Los Angeles and discussed areas of interest that were integrated with the RON, which is discussed below.

(ii) Energy and Economic Analysis, K. Stern, Chem Systems Inc. This activity consisted of further evaluations of biocatalyzed processes for acetone/butanol/ethanol (ABE). The base case was assessed in 1982, followed (1983) by a series of assessments where potential process advancements were included. These included vacuum fermentation, continuous fermentation, the Lignol Process (lignin to phenol, benzene and fuel, combined with ABE from cellulose), solvent extraction, a modified prehydrolysis and dual enzyme process, and an increased butanol tolerance process. Accomplishments: it was established that because the maximum product concentration is <2 wt%, separation energy requirements are so high (>20,000 btu/lb of solvents) that this is not currently a practical process. The problem is toxicity of product to the microorganism, which is common in many bioprocesses and can severely limit the rate of formation and concentration of products. As a result, the assessments of improved processes showed that increased butanol concentration tolerance is the most promising approach, which could decrease energy consumption by about 30%, and the selling price by 20%, to greatly improve the competitiveness of an ABE process.

(iii) Second Law Analysis, C. England, Engineering Research West The objective was to investigate energy conservation approaches that considered effects of the second law of thermodynamics, which generally leads to loss of energy because heat at a high temperature always flows in a direction toward a lower temperature, where it is often dissipated as waste heat. Accomplishments: thermodynamic analyses were applied to three separation processes: membrane separation, supercritical extraction and moving-bed chromatography, and it was concluded that energy efficiency is not as high as is often claimed for such processes because attention is focussed on the energy-efficient features of the process being considered. As a result, it is necessary to evaluate and modify such processes to maintain energy efficiency, for example by recovering waste heat and compression energy.

(iv) Research Opportunity Notice, J. Moacanin, R. Wilcox and J.D. Ingham, Jet Propulsion Laboratory This activity consisted of preparation and publication of a Research Opportunity Notice (RON) in the Commerce Business Daily to solicit expressions of interest from potential subcontractors in technical areas of concern to the Activity. Accomplishments: sixty seven responses to the RON were received from the chemical and biotechnology industries, universities, and research institutes, which expressed a strong interest in future proposals to the planned Request for Proposals (RFP).

C. Biocatalysis Project Description (1984)

In 1984 the Biocatalysis Research Activity was expanded and became the Biocatalysis Project. There were three technical work elements: Molecular Modeling and Applied Genetics, Bioprocess Engineering, and Process Design and Analysis. In FY 1984 there was also a Management work element that included work element coordination, planning and integration, technology transfer, and the Guidance and Evaluation Panel. The ECUT Program Manager was Dr. J.J. Eberhardt at the Department of Energy, and the Project Manager was Dr. M.N. Dastoor at the Jet Propulsion Laboratory.

Molecular Modeling and Applied Genetics

During FY 1984 this work element included three research activities in modeling and genetics: (i) Enzyme Reaction Models for Catalytic Processes, (ii) Chromosomal Amplification of Foreign DNA, and (iii) Enzyme Hyperproduction via Genetics.

(i) Enzyme Reaction Models for Catalytic Processes, W.A. Goddard, Caltech This research involved development of an enzyme catalysis model to describe the three-dimensional structure and molecular behavior in catalytic processes. The ultimate goal was to develop the capability to design new selective enzymes and enzymes for chemical conversions in nonaqueous media. Accomplishments: the force-field model for enzymes was refined, graphic software to visualize enzyme structure in three dimensions was developed, and calculations on the native form of the enzyme thermolysin showed excellent agreement with experimental structural studies.

(ii) Chromosomal Amplification of Foreign DNA, G.E. Bertani, Jet Propulsion Laboratory The purpose of this work was: (a) to demonstrate the feasibility of transfer of genetic material directly to the chromosome of a microorganism, and its amplification; and (b) to measure the genetic stability and compare it with a conventional genetically-engineered microorganism with genetic material carried by plasmids. Accomplishments: (a) chromosomal integration of appropriately engineered plasmids was confirmed by a physical method where DNA is cut and the fragments are analyzed, and (b) in two isolated strains, it appeared that duplication and amplification had occurred.

(iii) Enzyme Hyperproduction via Genetics, G.A. Nelson, Jet Propulsion Laboratory The objectives were to genetically alter the metabolic regulation of cellulolytic fungi to increase cellulase synthesis rates and to augment enzyme secretion rates to decrease separation costs. Accomplishments: (a) sixteen mutant strains of fungi were identified (from 1900) that had higher than normal activity, and (b) an improved genetic description of selected fungi was developed as a guide for future hyperproduction studies.

Bioprocess Engineering

Bioprocess Engineering consisted of two tasks: (i) Kinetics of DNA

Expression, and (ii) Control and Monitoring of Plasmids.

(i) Kinetics of DNA Expression, J.E. Bailey, Caltech The objective was to model genetically engineered microorganisms and determine how to control plasmid replication to develop stable, more efficient, selective biocatalysts and to establish methods to optimize genetic and environmental aspects of bioreactor processes. Accomplishments: (a) computer simulations of the effect of plasmid content on batch productivity with unstable recombinant cultures agreed with experimental results; (b) models based on molecular mechanisms suggested how to maximize gene expression, as indicated by promoter-utilization efficiency and other factors, which would maximize the amount of product formed.

(ii) Control and Monitoring of Plasmids, J.E. Bailey, Caltech Microorganisms can be tailored to produce chemical products or enzymes on demand by introducing genetic instructions in the form of plasmids into the microorganism. The objective of this work was to develop a method for plasmid monitoring. Accomplishments: (a) a computer and associated data analysis software were installed for real-time acquisition for two-parameter data to improve the level of detail and resolution between genetically different cells on the basis of flow cytometry measurements; (b) flow cytometry was used to determine the fraction of plasmid-containing cells and mean plasmid content to characterize the influence of deletions on the origin of replication.

Process Design and Analysis

This work element included two research tasks: Energy and Economic Analysis, and Software for Bioprocess Assessment.

(i) Energy and Economic Analysis, K. Stern, Chem Systems Inc This activity consisted of an evaluation of biocatalyzed processes for acetone/butanol/ethanol (ABE). The base case was assessed in 1982, followed (1983) by a series of assessments where potential process advancements were included. In 1984, several conceptual processes, where potential advancements were combined or added, were assessed to attempt to define a process with maximum energy efficiency. Accomplishments included the following. (a) Assessment of a case where continuous fermentation, Lignol, and dual enzymatic hydrolysis were combined showed a decrease in selling price of 6% and a decrease in energy of 14%, relative to the base case. (b) When the preceding were combined with an increased toxicity limit to 1.7 wt%, the resulting price and energy decreases were 30 and 46%. (c) When corn stover was used as feedstock instead of aspen wood, the calculated price decreased by about 10%.

(ii) Software for Bioprocess Assessment, E. Dunlop, Washington University The objective was to investigate the development of a planning and analysis computer program called BIOASPEN that was to be based on ASPEN, a program developed to assess synfuel processes.

In addition to capabilities for assessment of unit processes of most chemical operations, BIOASPEN was to take into account the characteristics of bioprocesses for more reliable assessments. Accomplishment: work was started in October, 1984.

Management

This activity included the Guidance and Review Panel, and the competitive procurement, "Advanced Bioprocess Concepts and Designs."

(i) Guidance and Evaluation Panel, J. Moacanin, Jet Propulsion Laboratory This consisted of a technical review board from universities, government agencies, and industrial companies. Accomplishments: the panel held a meeting at JPL to discuss the RON responses, and potential technical areas for emphasis in the RFP were suggested, such as advanced bioreactor concepts and improvements in sensors and process instrumentation.

(ii) Competitive Procurement: Advanced Bioprocess Concepts and Designs, M.N. Dastoor, R. Wilcox and J.D. Ingham, Jet Propulsion Laboratory A Request for Proposals (RFP) was released in May, 1984 to solicit proposals on generic, advanced bioprocess concepts and designs, particularly where molecular events would be related to macroscale behavior in the reactor. It was planned that these efforts would lead to eventual commercialization of large-scale bioprocesses. Accomplishments: (a) the RFP resulted in submission of 21 proposals, many with some form of cost sharing; and (b) evaluations of the proposals were completed in February, 1985.

D. Biocatalysis Project Description (1985)

In 1985 the Biocatalysis Project consisted of three technical work elements: Molecular Modeling and Applied Genetics, Bioprocess Engineering, and Process Design and Analysis; and a Management work element that included work element coordination, planning and integration, technology transfer, and the Guidance and Evaluation Panel. The ECUT Program Manager was Dr. J.J. Eberhardt at the Department of Energy, and the Project Manager was Dr. M.N. Dastoor at the Jet Propulsion Laboratory.

Molecular Modeling and Applied Genetics

During FY 1985 this work element included two research activities in modeling and genetics: (i) Enzyme Reaction Models for Biocatalysis, and (ii) Chromosomal Amplification of Foreign DNA.

(i) Enzyme Reaction Models for Catalytic Processes, W.A. Goddard, Caltech This research involved development of an enzyme catalysis model to describe the three-dimensional structure and molecular behavior in catalytic processes. The ultimate goal was to develop the capability to design new selective enzymes and enzymes for chemical conversions in nonaqueous media. Accomplishments: the enzyme carboxypeptidase A was simulated to confirm the model and to investigate the role of water in biocatalytic processes, and the origin of thermophilicity in thermolysin was studied.

(ii) Chromosomal Amplification of Foreign DNA, G.E. Bertani, Jet Propulsion Laboratory The purpose of this work was: (a) to demonstrate the feasibility of transfer of genetic material directly to the chromosome of a microorganism, and its amplification; and (b) to measure the genetic stability and compare it with a conventional genetically-engineered microorganism with genetic material carried by plasmids. Accomplishments: (a) bacterial strains containing multiple copies of a plasmid in their chromosomes were isolated and investigated; (b) it was confirmed that in the absence of selection, amplified chromosomal structures were unstable, but were more stable than when the plasmid was in the cytoplasm.

Bioprocess Engineering

Bioprocess Engineering consisted of three tasks: (i) Productivity of Recombinant Microorganisms, (ii) Immobilized Cell System for Continuous Efficient Biocatalyzed Processing, and (iii) Enzyme Catalysis in Nonaqueous Solutions.

(i) Productivity of Recombinant Microorganisms, J.E. Bailey, Caltech The objectives were to develop advanced methods for plasmid monitoring and to model genetically engineered microorganisms to determine how to control plasmid replication and develop stable, more efficient, selective biocatalysts; and to establish methods to optimize genetic and environmental aspects of bioreactor processes. Accomplishments: (a) computer simulations of

the effect of plasmid content on productivity were used to optimize batch reactor strategies; and (b) the effects of different types of flow reactor cascades were determined using the recombinant population model.

(ii) Immobilized Cell System for Continuous Efficient Biocatalyzed Processing, C.D. Scott, Oak Ridge National Laboratory To decrease energy consumption and capital equipment costs, it is necessary to increase productivities of fermentation chemical processes significantly. Therefore, the goals of this task were to enhance productivity and operability of a fluidized bed reactor system containing immobilized microorganisms, and to investigate bioreactor dynamics, including the formulation and investigation of kinetic properties of biocatalyst particles to lead to a better understanding of reactor behavior and control predictability. The forced flow of hydrocolloidal gels through a small nozzle with imposed vibration was shown to produce mono-dispersed biocatalyst beads containing microorganisms that are sufficiently small to be used in fluidized-bed bioreactors. The incorporation of inorganic oxide powder can be used to control density, and the oxide powder plus a more dense surface film can retard cell leakage. Accomplishments: (a) developed a method to immobilize biocatalytic microorganisms; (b) unsterilized feed was continuously converted (400 h) at a productivity of 243 g/(l-h) at 80% conversion and >95% yield; and (c) a continuous stirred reactor was operated using a commercial feed and nutrients, and immobilized catalyst.

(iii) Enzyme Catalysis in Nonaqueous Solutions, H. Blanch, U.C. Berkeley To permit the use of enzymes, in either free or immobilized form, to more broadly enter into the production of large scale chemicals, techniques for increasing the solubility and transport rate of organics at the site of enzyme action are needed. The approach was based on the use of a second organic liquid, water miscible or immiscible, in which the substrate or product is more soluble than in an aqueous system. Such systems are used currently for the enzymatic transformation of steroids. A second aspect of the use of enzymes in non-aqueous environments is the potential to run many reactions "backwards." In cases where water is a reaction substrate, its high activity in aqueous solution generally results in a shift of equilibrium to favor the normal products of the reaction. In a nonaqueous system such reactions may be forced in the reverse direction. Examples of this type of reaction include esterification, peptide synthesis and dehydration reactions such as urea formation from $(\text{NH}_4)_2\text{CO}_3$.

Two model systems were examined to develop generic technology for this effort. The first of these was the oxidation of cholesterol to the ketone. The second model system to be examined was the conversion of ammonium pyruvate to tryptophan. Accomplishments: (a) a two-phase system employing an organic solvent with the enzyme immobilized in an aqueous phase within microcapsules was developed for cholesterol conversion; (b) a membrane concept was defined with

the enzyme tryptophanase in a micellar or liquid membrane system, where reactant and product are transferred by an ionic carrier.

Process Design and Analysis

There were three research tasks: (i) Techno-Economic Assessment of Microbial Ammonia Production, (ii) Software for Bioprocess Assessment, and (iii) Bioprocess Synthesis, Integration and Analysis.

(i) Techno-Economic Assessment of Microbial Ammonia Production, K. Stern, Chem Systems Inc. This activity consisted of initiation of an evaluation of a biocatalyzed process for ammonia. Accomplishments: the base case was defined and preliminary process design for a bioprocess using the microorganism Anabaena was completed and evaluated.

(ii) Software for Bioprocess Assessment, E. Dunlop, Washington University The objective was to investigate the development of a planning and analysis computer program called BIOASPEN that was to be based on ASPEN, a previously developed assessment program. Accomplishments: (a) an assessment of the butanol bioprocess was initiated to define areas where program development was needed; (b) some costing and hydrolysis-step modules were developed for BIOASPEN.

(iii) Bioprocess Synthesis, Integration and Analysis, J.D. Ingham, Jet Propulsion Laboratory The purpose of this task was to derive or synthesize a series of candidate bioprocesses and systematically conduct relevant energy-economic analyses and comparisons for added-value commodity chemicals to determine potential for process technology transfer and commercial development. These bioprocesses could be modified to include projected research advances (e.g., genetically-engineered microorganisms, bioreactor modeling and verification, membrane development, and biocatalyst immobilization) for energy-economic comparative assessments. The four parameters that determine bioprocess economics and total energy requirements are cell density, productivity, product recovery and yield. Accomplishments: (a) an assessment of a process for ethyl acetate was initiated; (b) reactor modeling showed that fructose conversion rates are too low to allow high productivity in conversions to ethanol; and (c) a survey of experimental results indicated that high productivity (>100 g/l-h) is not obtained when product concentration exceeds 60 g/l.

Management

This activity included the Guidance and Review Panel; the competitive procurement, "Advanced Bioprocess Concepts and Designs"; the Industry Technology Transfer Workshop; and plans for a new task: "Biologically Effected Separation/Beneficiation."

(i) Guidance and Evaluation Panel, J. Moacanin, Jet Propulsion Laboratory This consisted of a senior technical review board. Accomplishments: the panel held a meeting at Caltech to discuss technology transfer. The main conclusion was that because of recent decreases in oil prices, a major challenge is to maintain and increase industrial interest in new bioprocess science and technology.

(ii) Competitive Procurement: Advanced Bioprocess Concepts and Designs, M.N. Dastoor, R. Wilcox and J.D. Ingham, Jet Propulsion Laboratory A Request for Proposals (RFP) had been released in May, 1984 to solicit proposals on generic, advanced bioprocess concepts and designs, particularly where molecular events would be related to macroscale behavior in the reactor. Accomplishments included the following. (a) New proposal evaluations were completed. (b) Five proposed contracts were initiated (from 21 proposers) including that with UCB (cf. Process Engineering, (iii), above). The other contractors were: Celanese, Cornell, Battelle Columbus, and Colorado State University.

(iii) Industry Technology Transfer Workshop, Washington University, St. Louis, MO The Workshop was held December 4-5 to review research activities and further industrial interest in Project activities.

(iv) Biologically Effected Separation/Beneficiation, Idaho National Energy Laboratory, Idaho Falls, ID The objective was to develop a detailed plan for future activities in biological separation and beneficiation. Accomplishments: (a) an overall strategy was developed to define a project to take advantage of biologically-effected separation to minimize industrial energy consumption; (b) a survey was initiated to determine energy aspects in the mining, chemical, food, and paper industries relevant to potential bioseparation processes.

E. Biocatalysis Project (1986-1990) and Catalysis and Biocatalysis Program (1990-1991)

From 1986 to 1990 the Biocatalysis Project consisted of three technical work elements: Molecular Modeling and Applied Genetics, Bioprocess Engineering, and Process Design and Analysis; and a Management work element that included work element coordination, planning and integration, technology transfer, and the Guidance and Evaluation Panel. In 1990, these elements were changed to Molecular Modeling and Catalysis-by-Design, Applied Microbiology and Genetics, Bioprocess Engineering, Separations and Novel Chemical Processes, Process Design and Analysis, and Management as part of the Catalysis and Biocatalysis Program. Until 1988 the ECUT Program Manager was Dr. J.J. Eberhardt at the Department of Energy, but was changed to Dr. Leonard Keay, who was Manager until March 7, 1991. The Jet Propulsion Laboratory Project Manager was Dr. M.N. Dastoor, followed by Dr. Gene R. Petersen from 1989-1991. The work described here was concluded in December 1991 with D.J. Boron as AICD Program Manager and J.D. Ingham as the closing task manager at JPL.

Molecular Modeling and Catalysis by Design

(i) Theory of Biocatalysis: Electron Transfer Proteins, D.N. Beratan, Jet Propulsion Laboratory In this research, the mechanisms of long-range electron transport in native and semi-synthetic proteins such as cytochrome c, blue copper, and iron-sulfur proteins, as well as electron transport in the photosynthetic reaction center, have been investigated in theoretical studies of biocatalysis. Because the transport events involve quantum mechanical tunneling of the electron from donor to acceptor, the chemical structural details of the intervening protein are critical. Results of this work include (a) new design rules for proteins with tailored electron delivery rates; (b) development of software that is widely used for the design of new protein systems; (c) suggestions of the crucial role of secondary structure on protein electron transport; and (d) formulation of a new theoretical method for calculating very weak electronic coupling in proteins.

(ii) Protein Engineering for Nonaqueous Solvents, F.H. Arnold, Caltech The industrial applications of biocatalysts have been severely limited by constraints on the solvent environment of proteins, which normally require an aqueous medium for effective operation. With the advent of convenient methods for altering the amino acid composition and for synthesis of entirely new proteins, it was decided to engineer proteins that would be effective in nonaqueous solvents. The success of a rational design procedure for constructing proteins to use in organic solvents depends on understanding relationships among various factors: amino acid sequence, secondary and tertiary protein structure, and activity and stability in nonaqueous solvents. The goal of this research

has been to define these relationships, by implementing an integral and iterative protein engineering approach based on the model hydrophobic protein crambin and other proteins.

The synthetic gene for crambin was expressed in *E. coli*. The gene was inserted into pKK223-3, containing the *tac* promoter. Crambin was expressed intracellularly. The protein produced in the bacteria was reduced and unfolded, but attempts to refold the recombinant crambin in a mixture of oxidized and reduced thiols succeeded. The genes for five variants of crambin were produced, including one with an additional disulfide bridge between residues 12 and 30. A set of design criteria for engineering proteins to be stable in nonaqueous solvents was proposed.

Subtilisin E was successfully expressed in the *E. coli* host strain, and purification was effected by removal of the periplasmic space from the host cells. Site-directed mutagenesis was used to construct subtilisin E mutants with improved stability in nonaqueous solvents, and subtilisin stability was improved by substituting specific amino acids in the protein. These results have demonstrated that it is possible to improve protein stability for applications in nonaqueous systems.

(iii) Catalysis-by-Design, Enzyme Reaction Models for Catalytic Processes, Center for Biocatalysis Design and Biotechnology Simulations, W.A. Goddard, Caltech This research involved development of an enzyme catalysis model to describe the three-dimensional structure and molecular behavior in catalytic processes, with the ultimate goal as the capability to design new selective enzymes and enzymes for chemical conversions in nonaqueous media. Accomplishments: two bacterial forms of the enzyme, Dihydrofolate Reductase were simulated to establish that the same active site surface was present and was constructed by different combinations of amino acids. Atomic level simulations were also completed for a type of artificial receptors known as Starburst dendrimers.

In 1990 the DOE Center for Biocatalysis Design and Biotechnology Simulations (DOE-CBD) was established in the Materials and Molecular Simulation Center (MSC) of the Beckman Institute at Caltech. An objective of the DOE-CBD is to have industrial collaborators that cost-share and participate in joint efforts at the MSC to attempt to resolve important problems in biotechnology. The first MSC workshop was held in July, 1990 on modeling polymers, biomolecules and crystal surfaces.

(iv) Modeling and Catalysis-by-Design: Simulations on Calcium Regulated and Redox Biocatalytic Systems, A. Redondo, P.J. Hay, and A.E. Garcia, Los Alamos National Laboratory The purpose of this work is to develop methods to model biocatalytic processes. Accomplishments: a method was developed for efficient calculation of reaction rates of biomacromolecules in solution, and

interactions of the effects of biopolymer and solvent using molecular dynamics were studied. The calculation of reaction rates was accomplished by solving a three-dimensional partial differential equation where the solution is the time-dependent probability of finding one molecule or atom at any point near a specified biomacromolecule.

Applied Microbiology and Genetics

(i) Metabolic Engineering, J.E. Bailey, Caltech The objectives are to determine the extent that cellular metabolism is sensitive to key individual metabolic activities, to develop experimental methods for investigation of metabolic perturbations, and to develop data for mathematical modeling and metabolic engineering. A Metabolic Pathway Synthesis (MPS) computer program was developed to predict the effects of adding or deleting enzyme activities in the cell environment, to classify pathways, and to extract information about metabolic regulation. MPS is also being used to identify genetic modifications that will amplify production of desired bioproducts. Cloned bacterial hemoglobin from Vitreoscilla was used to enhance growth and production in aerobic processes by increasing oxygen transfer, and the influence of plasmid presence and cloned gene induction on the activities of key metabolic enzymes was tested in Saccharomyces cerevisiae. Modeling of the kinetics of the ethanol production pathway indicated that ATP utilization rate and fructose-6-phosphate rate are critical controls for ethanol production and model predictions were verified by experiments using an inhibitor and cloned gene that affected these rates. Also a metabolic pathway model has been formulated to describe carbon flow and its regulation in the process for synthesis of biopolymer (polybetahydroxybutyrate, PHB) by A. eutrophus.

(ii) Chromosomal Amplification of Foreign DNA/Gene Fusion, G.E. Bertani, Jet Propulsion Laboratory The purpose of this work was: (a) to demonstrate the feasibility of transfer of genetic material directly to the chromosome of a microorganism, and its amplification; and (b) to measure the genetic stability and compare it with a conventional genetically-engineered microorganism with genetic material carried by plasmids. Accomplishments: Bacterial strains containing multiple copies of a plasmid in their chromosomes were isolated, but it was found that in the absence of selection, amplified chromosomal structures were unstable. Starting with a normal polymerase I, a new strain was isolated that carries the plasmid integrated and stable in the bacterial chromosome at the appropriate site.

(iii) Hyperproduction and Secretion of Polyphenol Oxidase, W.V. Dashek, Clark Atlanta University The objectives were: (1) to purify intracellular and extracellular polyphenol oxidases (PPO) from Coriolus versicolor maintained in liquid culture; (2) to determine if intracellular polyphenol oxidase is either de novo

synthesized or activated; and (3) to enhance PPO production, and scale up the liquid culture PPO bioprocess.

The approach used was to establish the time courses for the appearance of intracellular and extracellular polyphenol oxidase and to subsequently quantify oxidase specific activity, and to determine optimum growth conditions of C. versicolor for maximum production of polyphenol oxidase, including determination of cofactor requirements. Also, various concentrations of different inducers and genetic manipulation (mutagenesis, isolation of m-RNA's for in vitro translation, and gene cloning) were investigated to enhance production. For scale-up, optimum culture conditions were established, as well as rapid methods for PPO isolation and purification. For mutant production, treatment with nitrosoguanidine has resulted in C. versicolor cultures that had required increased resistance to a known inhibitor of PPO, diethylthiocarbamate. Following application of recDNA procedures, gel electrophoresis profiles of the modified plasmid pBR325 appeared to verify generation of a recombinant plasmid; E. coli cells transformed with the plasmid revealed resistance to ampicillin and tetracycline, but were sensitive to chloramphenicol. Experiments were carried out to verify insertion of C. versicolor DNA and to determine that PPO can be produced in E. coli cells.

Bioprocess Engineering

(i) Immobilized Cell System for Continuous Efficient Biocatalyzed Processing, C.D. Scott, Oak Ridge National Laboratory The goals of this task are to enhance productivity and operability of a fluidized bed reactor system (FBR) containing immobilized microorganisms, and to investigate bioreactor dynamics, including the formulation and investigation of kinetic properties of biocatalyst particles to lead to a better understanding of reactor behavior and control predictability. Mono-dispersed biocatalyst beads have been prepared. They contain microorganisms that are sufficiently small to be used in fluidized-bed bioreactors. The incorporation of inorganic oxide powder can be used to control density, and the oxide powder plus a more dense surface film can retard cell leakage. Experiments have been carried out where electrical conductivity probes were used to monitor gas phase fractions and determine effects of bubble size and flow rates in a reactor simulation. More recently, cellulose hydrolysis by cellulase has been demonstrated in an emulsion of dodecane in water, where more glucose was produced than in a control where dodecane was absent. Although dodecane tends to inactivate the enzyme, it has been demonstrated that a surfactant (Triton) protected the enzyme and caused a small enhancement in activity. An FBR process for n-butanol and acetone was developed, and work on enzyme catalysis in organic solvents was carried out. Experimental studies of hydrodynamics of larger scale (7.6 cm vs 2.5 cm internal diameter column) three-phase fluidized-bed bioreactors, followed by actual immobilized Z. mobilis fermentation have shown that gas

holdup was <3%, in agreement with model predictions for the larger columns. Because of the larger diameter relative to bubble size, coalescence and slugging were avoided, and the reactor was more efficient.

Polyethylene glycol, PEG,-dye-enzyme complexes were formed from several enzymes to increase solubility in benzene for reactions in organic solvents; the highest solubility was obtained for cytochrome C (0.8 mg/ml when 1 mg/ml was added). DEAE-Sepharose chromatography and fast protein liquid chromatography (FPLC) were used to separate the enzyme components of crude cellulase complex from T. reesei for studies of enzymatic activity in organic solvents, because more efficient conversion of cellulose to glucose would provide an inexpensive feedstock for production of commodity chemicals such as ethanol and its derivatives. An FBR was developed where two hydrodynamically separable solids were used for lactic acid production. One phase was the immobilized catalyst and the second solid phase was used to recover the product. The model for the study of hydrodynamics and kinetics of three-phase FBR's was completed and verified from experimental results.

(ii) Membrane Modifications for Alcohol Tolerant Bacteria, J.C. Linden, Colorado State Organic compounds presently derived from petroleum can also be obtained by fermentation of renewable raw materials. A problem common to many fermentations is the relatively dilute aqueous product produced, which requires high energy for product recovery. For example, the energy-economic impact of increasing the butanol concentration to 2.0 wt% in the fermentation process would decrease energy consumption (and associated costs) to less than half the amounts required using current bioprocesses. The goal of this task was to improve n-butanol production rate and concentration in a biocatalyzed process using biochemical modification of the cell membrane by incorporation of long-chain unsaturated fatty acids in the membrane to decrease butanol inhibition. Although cell membranes were modified, no significant improvements in n-butanol rates of production or product concentration were observed.

(iii) Molecular Hydrogen in Chemical Fermentations, P.C. Maxwell, Celanese Research, Celgene Glucose, which can be derived from renewable biomass, has two serious limitations as a fermentation raw material: selectivity to form specific end products is often low, and the variety of end products that can be made is limited. It had been found that if fermentation was conducted in the presence of hydrogen, the formation of oxidized coproducts was decreased so that selectivity was increased. In addition, the types of primary products formed may contain less oxygen. Two target fermentations were selected to demonstrate these concepts: fermentation of glucose to succinic acid which could demonstrate a desirable shift in product mix composition, and fermentation of glucose to 1,3-propanediol to demonstrate the ability to extend the range of end products. As a result of reorganizations, and changes

in the corporate objectives of the contractor, this task was discontinued before any significant results were obtained, and committed resources were minimized.

(iv) Multiphase Fluidized Bed Reactor, B. Allen, Battelle Columbus
The objective was to develop a fluidized bed bioreactor that combines the use of an immobilized biocatalyst (which consists of relatively large or dense fluidized particles) and circulation of a solid or insoluble solvent phase through the dense phase to adsorb or dissolve, and remove product. The latter entrained phase would then be sent to a separation unit to recover the product and regenerate the insoluble phase. Beads were prepared that contained bacterial cells for biocatalytic conversion that did not float, by making the beads smaller and effectively more dense. (Iron oxide was incorporated to increase density.) The mean distance of gaseous diffusion from inside the bead to the outside was reduced, which facilitated escape of gases and prevented buoyancy of the beads. The components to determine concept feasibility: synthesis and effective production of biocatalyst beads, determination of a nontoxic sorbent extractant, and evaluation of the extraction in a cold-flow system were all completed, but axial back-mixing was expected to be a potential problem. An economic assessment indicated that if a process based on this concept for n-butanol was developed, the cost of production could be decreased by about 40%. Industrial interests (including The Starch and Corn Growers Association, the State of Ohio, and Battelle Memorial Institute) were contacted and expressed interest in the multiphase fluidized bed reactor concept.

(v) Biocatalyzed Hydroxylation in Organic Solvents, A.M. Klivanov, MIT
The purpose of this research was to develop new methods for enzymatic oxidative catalysis in organic solvents, e.g., for selective oxidation of aromatic compounds to produce quinones and hydroxylated aromatic chemicals, and for peroxidase-catalyzed depolymerization of lignin, and potential polymerization of aromatic compounds.

The depolymerization of lignin was carried out to show that extracted lignins from milled wood or kraft pine can be successfully depolymerized using the same methods as for polyconiferyl alcohol. Also, milled wheat straw was delignified with a modified, dioxane-soluble peroxidase. No depolymerization took place in aqueous solutions, to show the advantage of enzymatic reaction in organic solvents. Studies were also completed on horseradish peroxidase-catalyzed reactions to polymerize phenols in organic solvents as an alternative to phenol-formaldehyde resins. Two inventions developed in this work have been licensed for commercial applications: Enzymatic Temperature Abuse Sensors; and Use of Dehydrated Enzymes for Measuring Gaseous Organic Compounds. Patents have not yet been issued for these inventions.

It was also shown that: (a) dehydrated alcohol oxidase successfully

converted alcohol vapor to acetaldehyde; (b) the dehydrated enzyme system was about 90 times more stable at 60°C than the aqueous system (as measured by retention of enzymatic activity for 180 min for the dehydrated system relative to 2 min for the aqueous system); (c) the dehydrated enzyme was most active when it was hydrated to a water content of ca 27%; and (d) incorporation of dehydrated catalase eliminated hydrogen peroxide inactivation, to allow complete conversion of alcohol to acetaldehyde. A new approach was implemented where an enzyme is lyophilized from an aqueous solution containing an appropriate ligand, followed by washing with an organic solvent to remove the ligand. The enzyme, while dry or in organic solvents, retains the conformation induced by the ligand, and has enhanced catalytic activity, altered specificity and greater stability. This phenomenon was investigated to determine its scope and generality, with the objective of developing a rationale for alteration of enzyme characteristics as desired, e.g., to increase and retain activity, or change specificity. Catalytic properties of biocatalysts can be improved by taking advantage of the high conformational rigidity of protein molecules in organic solvents, and by inducing long-term memory in proteins by ligand-protein lyophilization.

(vi) Integrated Biological-Chemical Process for Continuous Production of Methyl Ethyl Ketone and 1,3-Butadiene, G.T. Tsao and P.B. Beronio, Purdue U. The objective was to investigate and develop a bioprocess for continuous production of 2,3-butanediol as a precursor for production of 1,3-butadiene and methyl ethyl ketone. The technical approach included development and optimization of an immobilized cell bioreactor for the production of 2,3-butanediol and evaluation of solid sorbent technologies for product separation, including methods for sorbent regeneration. To ensure cell viability, NMR spectrometry was used to investigate bioenergetics and cell metabolism as bioreactor conditions were varied.

A method for making calcium alginate beads with immobilized biocatalyst was devised and used to make the diol, but without optimization the diol fermentation yield was only 11.8%; work was done to model the bioprocess; screening of adsorbents indicated that a 4Å molecular sieve is a good adsorbent for diol; and when nitrate was used to provide oxygen and nitrogen for fermentation, a yield of 45 g of 98% optically active diol from 126 g of glucose was obtained, along with 5 g of ethanol coproduct. Other accomplishments included optimization of growth and reactor conditions by manipulating nitrogen levels and mixing rates; measurement of kinetics in a CSTR reactor at low growth rates with variable oxygen uptake rates and carbon availability; and investigations of controlled nitrate feeding to produce optically active 2,3-butanediol.

(vii) Gas Phase Enzyme Biocatalytic Reactor, D.L. Wise, Northeastern U. This task was based on experiments of Barzana,

Klibanov, and others that showed that essentially dry enzymes can catalyze reactions in the vapor phase, such as conversion of ethanol vapor to acetaldehyde by alcohol oxidase. Most of the work was on developing and optimizing the immobilized oxidase enzymatic system and in optimization of reactor conditions. The best oxidase supports were Duolite ES-866 and Amberlite IRA-400 from Diamond Shamrock and Sigma Chemicals, respectively. Although oxidation occurred at up to 75°C, the optimum temperature was 25°C based on biocatalyst stability and product composition. An economic assessment of the enzymatic conversion of ethanol to acetaldehyde resulted in an estimated acetaldehyde price of \$1.64/kg, but assumptions and the basis for the assessment were not defined.

(viii) Enzyme Catalysis in Non-Aqueous Solutions and Enzyme Reactions in Reverse Micelles and Microcapsules, H. Blanch, U.C. Berkeley To permit more extensive use of enzymes in the production of large scale chemicals, techniques for increasing the solubility and transport rate of organics at the site of enzyme action are needed. The approach was based on the use of a second organic liquid, water miscible or immiscible, in which the substrate or product is more soluble than in an aqueous system. Such systems are used currently for the enzymatic transformation of steroids. A second aspect of the use of enzymes in non-aqueous environments is the potential to run many reactions "backwards." In cases where water is a reaction substrate, its high activity in aqueous solution generally results in a shift of equilibrium to favor the normal products of the reaction. In a nonaqueous system such reactions may be forced in the reverse direction. Examples of this type of reaction include esterification, peptide synthesis and dehydration reactions such as urea formation from $(\text{NH}_4)_2 \text{CO}_3$.

Two model systems were examined to develop generic technology for this effort. The first was the oxidation of cholesterol to the ketone. The second model system examined was the conversion of ammonium pyruvate to tryptophan. Earlier accomplishments included the following. (a) A two-phase system employing an organic solvent with the enzyme immobilized in an aqueous phase within microcapsules was developed for cholesterol conversion. (b) A membrane concept was defined with the enzyme tryptophanase in a micellar or liquid membrane system, where reactant and product are transferred by an ionic carrier. In subsequent work, two other enzymes (alpha-chymotrypsin, and horse liver alcohol dehydrogenase, LADH) were investigated in reverse micelles. (Reverse micelles are small aqueous droplets stabilized by a surfactant in a solvent such as isooctane). It was determined that the chymotrypsin enzyme can show enhanced activity in reverse micelles relative to aqueous solutions, but LADH showed reduced activity which was attributed to changes in structure, on the basis of fluorescence and EPR measurements. It was also found that surfactants used for micelle formation could cause deactivation. The hydrolysis of protein in reverse micellar solutions by chymotrypsin was investigated to determine conversion kinetics.

(ix) Immobilized Enzymes in Organic Solvents, H. Zemel, Allied Signal Research and Technology The objective was to investigate lipase transesterification of palm oil in petroleum ether, as a low cost alternative to cocoa butter, which is widely used in the confectionery industry. A uniform theory has been formulated which provides consistent explanations for many observations in enzymatic systems with variable water content, provides prediction of optimum water contents in the operation of enzymes in organic solvents, and provides a clearer view of research needed to develop enzyme systems for commercial process technology. Results of this work show that commercial applications are feasible with systems in organic solvents with less than 1% water as long as one treats such systems as extensions of aqueous media. The catalysis occurs in water, but with the solubility advantages of an organic solvent system. The optimization of reactivity is reduced to studies of the preferred pH, ionic strength, and choosing a solvent, all with minimal inhibition in an aqueous phase.

Separations and Novel Chemical Processes

(i) Separation by Reversible Chemical Association, C.J. King, U.C. Berkeley The objective of this task is to examine and evaluate the use of reversible chemical association, or complexation, with organic agents as a method for separating polar organic substances from dilute aqueous solutions, e.g., bioprocess product or waste streams. The goal is to obtain sufficient understanding of underlying chemical, equilibrium, and transport behavior to enable rational selection of separating agents, methods of regeneration, and methods of implementation, as well as rational conceptual design and economic evaluation. In the production of carboxylic acids, processes such as fermentation yield low concentrations of carboxylic acids in an aqueous multicomponent solution. The subsequent separation, purification, and concentration of the carboxylic acids is often difficult and energy intensive.

Data have been obtained for co-extraction of water when methyl isobutyl ketone (MiBK) is used as a diluent with Alamine 336 for extraction of succinic acid. Equilibrium data have been obtained for extraction of succinic acid by Alamine 336 with an alkane diluent. Data for three diluents (the alkane, chloroform, and methyl isobutyl ketone, MiBK) were compared and interpreted. Spectroscopic methods were used for interpretation of complexation in both carboxylic acid/amine and alcohol/phenolics extraction systems. It was found that for adipic, fumaric and succinic acids there is an increase in solubility in the extractant when water was coextracted, and that subsequent evaporation of the water caused precipitation of the product for relatively easy recovery. It was calculated that this method requires only about 25% as much energy as an efficient evaporation system. A method was partly developed for extraction of glycols as complexes with boron compounds such as 3-nitrophenylboronic acid.

(ii) Biological Separation of Phosphate from Ore, R.D. Rogers, Idaho National Engineering Laboratory The phosphate industry utilizes about 0.3 quads/yr for the separation of phosphate from apatite ore, or rock phosphate (RP). Therefore this task has been directed toward development of bioprocessing for solubilization and separation of phosphate from ore. The specific objectives of the research were formulated to: (1) define a microbiological system that will extract phosphate from its ore; (2) develop a basic understanding of the biochemical mechanisms involved; and (3) through the use of modern biotechnology develop a bioprocessing system for transfer to the phosphate industry. If a more efficient recovery process can be developed, it could also be applied to phosphate mine waste because ore containing <26% phosphorus is currently considered waste and is used as mine backfill.

The approach has been to gain an understanding of the biochemical interactions that cause the microbial release of phosphate from its ore. This task was initiated by screening microorganisms obtained from areas of high phosphate content (i.e., phosphate mines, process waste streams, fertilized agricultural lands, etc.). Those organisms that were positive for the desired trait (phosphate solubilization) were subjected to further biochemical studies. It was found that RP solubilization was highest when acidification and chelation were enhanced by bacterial interactions. INEL collaborated with the TVA National Fertilizer Center and the Phosphate Chemicals Division of the FMC Corporation, to use a computer model (MINEQL) to model apatite solubilization and to investigate process development. Results indicated that low-cost heap leaching should be practical for phosphate recovery from low-grade RP or mine waste.

(iii) Electrocatalytic Study of Ammonia Syntheses and Methane Dimerization in Solid Electrolyte Cells, M. Stoukides, Tufts University High temperature proton conductors are solid state materials that can be used as heterogeneous catalysts to electrochemically modify hydrogenation and dehydrogenation reactions. Useful chemicals and electrical energy could be generated at the same time if the electrolytic cell is operated as a fuel cell. First, it was necessary to test various materials as proton conductors. It was found that an electrolyte made by mixing powders such as strontium carbonate, cerium dioxide and ytterbium, followed by slow heating to 1450°C, calcining, and pressure molding, could be used in a special cell to conduct protons. In this work, ammonia synthesis and conversion of methane to ethylene were proposed as examples of useful dehydrogenation and hydrogenation reactions, respectively, to be investigated. Many catalyst systems were prepared and used to convert methane at 900-1000°C, but at high methane concentrations, carbonaceous deposits formed and lowered catalyst activity and C₂ selectivity. The maximum selectivity found over Fe was 23%, and the predominant product was ethylene.

(iv) Aqueous Two-Phase Extractive Fermentation for Bulk Chemicals, W.A. Weigand, University of Maryland The objective was to investigate systems where two aqueous phases, each containing a different polymer that make the phases immiscible, were used to minimize product rate inhibition by product extraction into one phase as it was formed in the other. The approach included studies to: (a) determine the optimal polymer compositions of the two phase system in terms of chemical and physical properties; (b) develop a method for establishing the casual relationship between various fermentation variables and the productivity of butanol-acetone, where the key metabolic intermediate, NADH, was used as an indicator of metabolism of C. acetobutylicum in the two phase system and in ordinary batch fermentation; (c) investigate the biocompatibility between the aqueous polymer solutions and C. acetobutylicum and compare operation of the two phase system with ordinary batch fermentation; and (d) determine the technical feasibility and a technological estimate of the potential industrial applicability of new bioprocesses based on the preceding activities, where product inhibition is minimized and product recovery is facilitated by utilization of an aqueous two-phase extraction system. Results indicated that this type of process is feasible, but specific problems (that may be resolved by further work) are relatively inefficient product extraction and resulting difficulties in isolating the products at high yields.

Process Design and Analysis

(i) Bioprocess Synthesis, Integration and Analysis: A Biological and Chemical Process Computer Model, BCP1, J.D. Ingham, Jet Propulsion Laboratory The purpose of this task was to: (i) derive or synthesize a series of candidate bioprocesses; (ii) systematically, for added-value commodity chemicals, conduct relevant energy-economic analyses and comparisons to determine potential for process technology transfer and commercial development; and (iii) develop a user-friendly computer model, BCP1, for process assessments. Bioprocesses may be modified to include projected research advances (e.g., genetically-engineered microorganisms, bioreactor modeling and verification, membrane development, and biocatalyst immobilization) for energy-economic comparative assessments. Previous accomplishments included the following. (a) An assessment of a process for ethyl acetate was completed. (b) Reactor modeling showed that fructose conversion rates are too low to allow high productivity in conversions to ethanol. (c) A survey of experimental results indicated that high productivity (>100 g/l-h) is usually not obtained when product concentration exceeds about 60 g/l. Assessments of processes for ethanol and citric acid using column bioreactors with immobilized cells as fluidized-bed biocatalysts were also carried out. It was found that the effect of increased productivity is advantageous, but the relative advantages for various processes are strongly dependent on product concentration and the ratio of fermentor capital-related costs to total capital-related costs for the

specific bioprocess. Therefore, as productivity is increased, product concentration should be maintained above about 70 g/l, and increases in productivity above critical levels do not necessarily provide significant economic advantages.

The current primary objective is to develop a computer model (BCP1) that can be used to determine the energy requirements and approximate investment and other costs for chemicals that can be produced by various biological and chemical processes. There are several commercial computer-aided design (CAD) programs, such as ASPEN, that can be used for detailed chemical process assessments; however, their primary purpose is to develop detailed process designs for specific chemical plants. They are not normally available at moderate cost, are not user-friendly, and require special computer capabilities; such as a 386 computer with 6-16 megabytes of expanded memory. In addition, as CAD simulators have been improved, computer requirements have been extended to accommodate larger data bases and increased complexity, and this trend is expected to continue. A preliminary version of the BCP1 computer model was used to estimate fermentation costs for ethanol, and for comparisons for an ethyl acetate process with an ASPEN simulator. A series of specific fermentation processes and chemical processes (such as hydrogenation, dehydration, esterification) is included in the BCP1 model for selection from menu lists, and the selected type is modified as appropriate for an assessment. For a specific reaction of each type, default values for numerous parameters and variables, such as stoichiometric factors, properties, rates, concentrations, materials costs, and economic values, are displayed and can be changed during the assessment run, or in the program, to create a new assessment. Mass and energy balances are provided and costs are computed on the basis of a factored estimate. A large number of known processes will be included and verified to ensure that cost estimate protocols are reliable. After any base-case estimate, each additional assessment (where numerous process variations can be incorporated) may be completed within a few minutes. Since only the subprograms for each specific assessment are put into the computer memory as needed (and are later automatically deleted), expanded memory or other special computer capabilities are not needed. The current version consists of a series of interactive menu-driven subprograms that run consecutively. Process-specific subprograms are automatically preselected when the process name is selected from the first menu. The completed program will include a user-accessible library of equipment types that are automatically scaled according to process flow rates, with appropriate cost factors for materials, year of construction, and specific process requirements. The purpose of the model is to provide rapid, reliable assessments of process concepts rather than specific equipment or detailed design capabilities. As a result, some details are omitted in the interest of user convenience and speed, but reliability of the final results that are important (such as capital costs, and other contributions to production and sales

costs, as well as energy requirements) is maintained by comparisons with previous or more detailed assessments. Recent accomplishments are: (a) completed process synthesis and a successfully consistent comparison of the BCP1 model with an ASPEN simulation for ethyl acetate, where bioprocess ethanol is chemically converted to ethyl acetate; (b) a flowsheet subprogram has been developed and integrated into a new version of the computer model; (c) a rigorous fractional distillation subprogram for nonideal binary solutions has been developed and integrated with the model; (d) to minimize memory requirements, improvements have been made to delete preceding subprograms after information or procedures are no longer needed (but with retention of all necessary property and variable values).

(ii) Techno-Economic Assessment of Microbial Ammonia Production, K. Stern, Chem Systems Inc. This activity consisted of an evaluation of a biocatalyzed process for ammonia. The base case was defined and preliminary process design for a bioprocess using the microorganism *Anabaena* was completed. The process design was based on utilization of large lagoons for this process, and was considered to be of relatively little interest because of the extensive capital investment required. Although ammonia is a low-cost, very high volume product, current processes are energy-intensive. Since the feedstock is methane, if there was a shortage of natural gas, ammonia would probably be made by current chemical processes from methane produced by biological processing.

(iii) Assessment of the Role of Biotechnology in Commodity Chemical Production, K. Stern, Chem Systems Inc. The goal of this task was to evaluate the current perspectives of major commodity chemical producers and biotechnology-oriented companies toward the goal of bioproduction of commodity chemicals, and to identify the key issues that these companies see as influencing potential industrial applications of bioprocesses. The approach was to determine which companies would be interested in attendance and participation in a roundtable conference on bioprocess chemical production; compile a list of candidate chemicals and select the most promising; and establish the basis for process evaluations (in cooperation with participating companies), followed by technoeconomic assessments of bioprocesses for selected chemicals. Then a conference was convened to arrive at an industry position on the feasibility of increased commodity chemical production by bioprocesses, including identification of any major technical or economic obstacles to expanding such applications.

The chemicals selected for assessments were propylene oxide and caprolactam. It was concluded that an Exxon bioprocess for propylene oxide could be economically competitive with current commercial processes if it can be demonstrated at a reasonable scale of operation. The process is based on the use of immobilized methylotropic microorganisms to enzymatically catalyze the oxidation of propylene to the epoxide, but the process also requires methane and methanol for cell production and regeneration,

and is very energy intensive. Similar results were obtained in assessments of processes for caprolactam. Adiponitrile (which can be made by reaction of butadiene with hydrogen cyanide) is enzymatically converted to 5-cyanopentanoic acid which is then reacted with hydrogen to make 6-aminocaproic acid, which can be dehydrated to epsilon-caprolactam. This process should be economically competitive after demonstration at a reasonable scale, but the energy required is higher than for the current commercial process. On June 21-22, 1988, an industry discussion group was convened in Washington to consider the role of biotechnology in US commodity chemical production. Some major comments at this meeting suggest that for production of commodity chemicals by biocatalyzed processes, expectations have been limited by inadequate separation technology and biocatalyst stability, and institutional inertia to retain conventional chemical processes. Recommendations for future work included: improvements in separation processes, biocatalyst stability, cofactor regeneration, development of biomimetic systems, more active technology transfer efforts, and increased development and enhancement of assessment methods for more meaningful comparisons of process economics. Representatives from DOE, Celgene, AMGEN, Genex, Dow Chemical, PPG Industries, E.I. Du Pont and Eastman Kodak attended the meeting, in addition to those from JPL and Chem Systems.

(iv) Economic Evaluation of Potential Bioprocesses, R.M. Busche, Bio-En-Gene-Er Associates, Inc. The goal of this task was to evaluate the economics of specific bioprocess concepts to determine if they would be economical. Concepts evaluated included: an N-butanol process where the product was extracted by a hypothetical solvent to reduce product inhibition; use of recombinant aerobes for specialty chemicals; the use of Z. mobilis for ethanol production; and recovery of acetic acid by solvent extraction. It was found that the cost advantage for the Z. mobilis bioprocess is about 5%, and that use of recombinant aerobes could provide significant advantages if genetically-engineered aerobes had other desired characteristics (in addition to enhanced oxygen transfer). It appears that important advantages of extractive processes depend on the availability of currently hypothetical, idealized solvents with an unusual combination of characteristics. Because extraction characteristics (such as selectivity for specific products) depend on gross or general features, such as polarity, or oxygen content, it is not likely that such solvents can be synthesized or discovered. For example, for alcohol or acid extraction (in the absence of reversible association, as in work by C.J. King, described above) the extractant must be polar, but polar solvents are at least partly soluble in water, and would be as difficult to recover as the product. As a result, the proposed extraction process may not be intrinsically energy-intensive, but would not be economically viable because of solvent loss and high waste-stream pollution.

Management

This activity included a survey of biocatalysis programs (1987) and a technology transfer activity (1990) as well as conventional and necessary management functions, such as technical and contract management, reporting and presentations, and prioritization and allocation of resources.

(i) Biocatalysis: Federal Programs and Information Resources, O. Zaborsky, OMEC International The purpose of this task was to identify and describe programs and information resources on biocatalysis for use in research planning. A survey of federal agencies was completed and descriptions were developed on biocatalysis activities and information or data sources, followed by preparation of a report. (At the present time similar, more timely information is provided in, for example, "Industrial Energy Technology", S.C. Hicks and J.D. Bales, editors, available from the Office of Scientific and Technical Information, US Department of Energy, PO Box 62, Oak Ridge, TN 37831).

(ii) Technology Transfer, (J.S. Tuan, Bernard Wolnak and Associates In May, 1990, a conference on "The Industrial Use of Enzymes: Technical and Economic Barriers" was held in Chicago. Also a survey was completed on Project enzyme research and technology. Five companies expressed significant interest in future exchange of information: Biodesign, Inc; Bioprobe International Inc; Catalytica; Pharmacia LKB Biotechnology and Sepragen.

Table 1 Summary of Biocatalysis Research Projects or Tasks			
Principal Investigator Institution	Project or Task Title	Duration, Months	Contract Amount, \$K
Allen, B. Battelle Columbus	Multiphase Fluidized Bed Reactor	36	473
Arnold, F. Caltech	Proteins Engineered for Nonaqueous Solvents	52	586
Bailey, J.E. Caltech	Metabolic Engineering	96	1,467
Bailey, J.E. Caltech	Kinetics of Recombinant Microorganisms	96	464
Beratan, D.N. JPL	Electron Transfer Proteins	48	790
Bertani, G. JPL	Chromosomal Amplification	96	627
Blanch, H.W. University of CA, Berkeley	Enzyme Catalysis in Nonaqueous Solutions	74	395
Busche, R.M. Bio-En-Gene-Er Associates	Economic Evaluation of Potential Bioprocesses	18	70
Campbell, J.A. Caltech	Chromosomal Amplification	48	102
Dashek, W.V. Clark Atlanta University	Overproduction and Enhanced Secretion of Enzymes	36	198
Di Stefano, S. JPL	Decarboxylation	12	45
Dunlop, E. Washington University	System for Biotechnology Assessment	18	76
England, C. Engineering Research West	Second Law Analysis	24	90
Goddard, W.A. Caltech	Enzyme Models, Catalysis-by-Design	96	1,305

Table 1 Summary of Biocatalysis Research Projects or Tasks			
Principal Investigator Institution	Project or Task Title	Duration, Months	Contract Amount, \$K
Goddard, W.A. Caltech	Materials Simulation Center	24 (and future work)	681
Gray, H.B. Caltech	Electron Tunneling in Engineered Proteins	45	94
Hay, J.P. LANL	Modeling and Catalysis-by-Design	52	1,050
Ingham, J.D. JPL	Process Synthesis, Assessment and Integration	38	385
King, C.J. LBL, UCB	Separations by Reversible Chemical Association	36	430
Klibanov, A.M. MIT	Catalyzed Hydroxylation of Aromatic Compounds in Organic Solvents	72	551
Linden, J.C. Colorado St	Membranes Modified for Alcohol Tolerant Bacteria	24	86
Lonsdale, H.K. Bend Research	Membrane Technology	9	18
Maxwell, P.C. Celanese Research Corp	Molecular Hydrogen in Chemical Fermentations	32	202
Nelson, G.A. JPL	Cellulase Hyperproduction	24	225
Rogers, R.D. INEL	Biological Separation of Phosphate from Ore	36	804
Scott, C.D. ORNL	Immobilized Cell System for Biocatalytic Processing	38	804
Shuler, M.L. Cornell University	Multimembrane Reactor for Chemical Production	24	352

Table 1 Summary of Biocatalysis Research Projects or Tasks			
Principal Investigator Institution	Project or Task Title	Duration, Months	Contract Amount, \$K
Stern, K. Chem Systems Inc.	Assessment of the Role of Biotechnology in Chemical Production	9	85
Stern, K. Chem Systems Inc.	Techno-Economic Assessments of Bioprocesses	28	136
Stoukides, M. Tufts University	Electrocatalysis: Ammonia Synthesis and Methane Dimerization	12	50
Tsao, G.T. Purdue University	Integrated Biological Chemical Process for Methyl Ethyl ketone	24	183
Voecks, G.E. JPL	Ammonia Synthesis	12	60
Warren, L.F. Rockwell International	Electrocatalysis	12	22
Weigand, W.A. University of Maryland	Aqueous Two-Phase Extractive Fermentation for Chemicals	12	80
Wilcox, R.E. JPL	Technical Planning: RON, RFP, Multi-Year Plans	48	670
Wise, D.L. Northeastern University	Gas-Phase Enzyme Bioreactor	33	206
Zemel, H. Allied-Signal Research	Immobilized Enzymes in Organic Solvents	39	241
None specified Critical Fluid Systems	Assessment of Critical Fluid Extractions	6	15

SECTION III

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M.L. Shuler

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(T. Cho and M.L. Shuler, Assigned to Cornell Research
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Foundation, Inc.), U.S. Patent 4861483 (August 29, 1989).

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Advanced System for Production of Biocatalyst Beads. DOE
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Biosorption Beads for Removal of Dissolved Metals from Aqueous
Streams. DOE Docket #S-64,986 (October 1986).

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Regeneration of Carboxylic Acid Extracts by Selective Removal
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Acid (submitted 1990).

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Briefs 13, 44 (1989); patent filed by NASA. A FORTRAN program to determine electron tunneling pathways in proteins was submitted to COSMIC, the NASA software center 12/89.

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Enzymatic Temperature Abuse Sensors; patent application, MIT Case #4388-86.

SECTION IV

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